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Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant *ABCC8/KCNJ11* mutations

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Abstract

Aims/hypothesis Dominantly acting loss-of-function mutations in the *ABCC8/KCNJ11* genes can cause mild medically responsive hyperinsulinaemic hypoglycaemia (HH). As controversy exists over whether these mutations predispose to diabetes in adulthood we investigated the prevalence of diabetes in families with dominantly inherited ATP-sensitive potassium (K_{ATP}) channel mutations causing HH in the proband.

Methods We studied the phenotype of 30 mutation carriers (14 children and 16 adults) from nine families with dominant *ABCC8/KCNJ11* mutations. Functional consequences of six novel missense mutations were examined by

reconstituting the K_{ATP} channel in human embryonic kidney 293 (HEK293) cells and evaluating the effect of drugs and metabolic poisoning on the channels using the ^{86}Rb flux assay.

Results The mutant channels all showed a lack of ^{86}Rb efflux on exposure to the channel agonist diazoxide or metabolic inhibition. In the families, dominant *ABCC8/KCNJ11* mutations were associated with increased birth-weight (median + 1.56 SD score [SDS]). Fourteen children had HH and five adults were reported with HH or hypoglycaemic episodes (63%). Progression from hypoglycaemia to diabetes mellitus occurred in two individuals. Eight adults had a history of gestational diabetes in multiple pregnancies or were diabetic (diagnosed at a median age of 31 years). Within these families, none of the 19 adults who were not carriers of the *ABCC8/KCNJ11* mutation was known to be diabetic.

Conclusions/interpretation The phenotype associated with dominant *ABCC8/KCNJ11* mutations ranges from asymptomatic macrosomia to persistent HH in childhood. In adults, it may also be an important cause of dominantly inherited early-onset diabetes mellitus.

Keywords Diabetes mellitus · Hyperinsulinism · Hypoglycaemia · K_{ATP} channels

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Abbreviations

CHI	Congenital hyperinsulinism
DM	Diabetes mellitus
GDM	Gestational diabetes mellitus
HH	Hyperinsulinaemic hypoglycaemia
K_{ATP} channel	ATP-sensitive potassium channel
Kir6.2	Inward rectifier $K^{(+)}$ channel Kir6.2
SDS	Standard deviation score
SUR1	Sulfonylurea receptor 1

Introduction

Hyperinsulinaemic hypoglycaemia (HH) occurs because of inappropriate secretion of insulin by the pancreatic beta cells. The congenital forms of HH (CHI) occur because of defects in key genes involved in insulin secretion [1]. The commonest and most severe forms of CHI are due to mutations in the *ABCC8* and *KCNJ11* genes [2, 3], encoding the sulfonylurea receptor 1 (SUR1) and inward rectifier K(+) channel Kir6.2 (Kir6.2) subunits of the ATP-sensitive potassium (K_{ATP}) channel, respectively. The K_{ATP} channels play a pivotal role in glucose-stimulated insulin secretion and couple glucose metabolism to membrane electrical activity and insulin release in the pancreatic beta cells [4].

Recessively inherited inactivating *ABCC8/KCNJ11* mutations usually cause medically unresponsive disease. The molecular basis of the CHI observed in these patients involves defects in the biogenesis and turnover of K_{ATP} channels, in the trafficking of channels to the plasma membrane and alterations in the open-state frequency through changes in nucleotide sensitivity [5–8].

Dominant *ABCC8/KCNJ11* mutations are less frequently reported and generally cause mild medically responsive HH [9–12] or in rare cases, severe unresponsive HH [13]. Two reports suggest that medically responsive HH due to a dominantly inherited *ABCC8* mutation may progress to diabetes mellitus (DM) in later life [9, 12, 14]. However, this was not supported in a recent case series where only 4 out of 29 adult mutation carriers developed DM [11]. Hence, it is not clear whether dominantly acting mutations in the genes that cause diazoxide-responsive HH in childhood are associated with later development of DM in adulthood.

In this study, we present the phenotype of eight different heterozygous *ABCC8/KCNJ11* mutations in nine families and report the prevalence of DM in the adult mutation carriers. We show that dominant mutations can cause a variable phenotype ranging from asymptomatic macrosomia to transient/persistent HH as well as progression to DM in later life.

Methods

Patients Nine families with dominant K_{ATP} channel mutations were studied. The probands are a subgroup of children referred to the tertiary Hyperinsulinism Service at Great Ormond Street Hospital NHS Trust, London, UK. They include those children with HH who were diazoxide responsive and were identified to be heterozygous for a K_{ATP} channel mutation. The diagnosis of HH was based on diagnostic criteria described previously (i.e. inappropriately

elevated insulin concentrations at the time of hypoglycaemia with corresponding low concentrations of plasma β -hydroxybutyrate and fatty acids). Diazoxide responsiveness was defined as the ability to maintain normoglycaemia without the support of intravenous glucose. Clinical information (birthweight, age at presentation, treatment details of HH) was collected from the case notes and the referring clinicians. Family history was specifically explored with regards to symptoms of hypoglycaemia and presence/absence of DM and phenotypic details of individuals affected by hypoglycaemia/DM (birthweight, age of presentation, treatment details) was collected. The Office for National Statistics (ONS) classification was used to categorise ethnicity [15]. The study was approved by the regional ethical committee and written consent was obtained from the families.

Genetic analysis Genomic DNA was extracted from peripheral leucocytes using standard procedures. The single exon of the *KCNJ11* gene and the 39 exons of *ABCC8* were amplified by PCR using previously reported primers [16]. Single-strand sequencing was carried out using standard methods and an ABI3730 capillary sequencer (Applied Biosystems, Warrington, UK) and sequences were compared with the published sequences (NM_000525 and NM_000352.2 [which incorporates the alternatively spliced amino acid in exon 17 {L78224}]) using Mutation Surveyor 3.24 software (SoftGenetics, State College, PA, USA). Following identification of a mono-allelic K_{ATP} channel mutation in the proband, parents were tested to establish the mode of inheritance. Microsatellite analysis (PowerPlex 16 System, Promega, Southampton, UK) was undertaken to confirm family relationships when a de novo mutation was identified. Genetic analysis was then offered to the siblings of the proband (parental consent obtained) and to the siblings and parents of the parent identified as heterozygous for the mutation. Co-segregation studies were then undertaken with the available phenotypic data and results of the genetic analysis.

Functional analysis of mutant channels Functional analysis of the novel *ABCC8* mutations (A1508P, A1537V, L1431F, L1390R and Q1459E) and the *KCNJ11* mutation (I284del) was carried out. Mutations were introduced in hamster *Sur1* (also known as *Abcc8*) cDNA and mouse *Kir6.2* (also known as *Kcnj11*) cDNA using the Quik-change site-directed mutagenesis kit (Agilent Technologies, Stockport, UK). Mutant K_{ATP} channel was reconstituted in human embryonic kidney 293 (HEK293) cells by transfection using FuGene HD (Roche, Burgess Hill, UK) according to the manufacturer's instructions. Enhanced green fluorescent protein, 50 ng, was also co-transfected to determine transfection efficiency.

Following successful transfection, an ^{86}Rb flux assay was used to determine functionality of the reconstituted channels as described previously by Muzyamba et al. [17]. The ^{86}Rb efflux assay is based on the principle that when cells are loaded with rubidium, its distribution between intracellular and extracellular spaces is an indicator of channel activity as ^{86}Rb is similar in size and charge to K^+ and is handled by the channels in a manner similar to K^+ . Briefly, 24 h after transfection cells were incubated overnight with minimum essential medium (MEM) containing ^{86}Rb (0.037 MBq/ml) at optimum conditions (5% CO_2 , 37°C). The assay medium was then aspirated and the cells were washed twice with 2 ml HEPES buffered NaCl (HBS) (10 mmol/l HEPES, 10 mmol/l glucose, 130 mmol/l NaCl, 7 mmol/l KCl, 2 mmol/l CaCl_2 and 1 mmol/l MgCl_2 , pH 7.4) [17]. The cells were then incubated with 2 ml of HBS medium with or without channel stimulants and/or inhibitors. The following conditions were used: control cells (no drugs); cells treated with 100 $\mu\text{mol/l}$ diazoxide; cells treated with 100 $\mu\text{mol/l}$ diazoxide plus 10 $\mu\text{mol/l}$ glibenclamide; cells treated with 2.5 mmol/l NaCN (sodium cyanide) and 20 mmol/l 2-deoxy-D-glucose; and cells treated with 2.5 mmol/l NaCN, 20 mmol/l 2-deoxy-D-glucose and 10 $\mu\text{mol/l}$ glibenclamide. The concentrations of the drugs stated have been shown to be effective on this cell line [17]. The supernatant fraction was aspirated after 5 min into scintillation vials for counting and the cells were then lysed with 2% Triton/HBS solution. Cherenkov radiation in the supernatant fraction and cell lysates was measured and the efflux was calculated as a percentage relative to the total amount of radioactivity incorporated. The data are presented as mean \pm SEM. Significant differences were tested by employing the one-way ANOVA and Bonferroni post-test. Significant differences are reported as: $*p \leq 0.05$, $**p \leq 0.01$ and $***p \leq 0.001$.

The level of mutant protein produced in the transfected cells was detected using western blotting. The antibody anti-SUR1 NBD2 was raised in sheep to the peptide epitope ETLLSQKDSVFASFVRADK (University of Dundee, Dundee, UK) and subsequently used as described previously by Muzyamba et al. [17]. An anti-sheep IgG secondary antibody (Sigma, Poole, UK) linked to horseradish peroxidase and the ECL detection kit (GE Healthcare, Amersham, UK) were used to detect protein production.

OGTT and BMI The adult mutation carriers were divided into those with a history of DM (gestational or other, $n=8$) and those without ($n=8$). A 2 h OGTT was conducted in the relatives of adult mutation carriers who had no history of DM to ensure that undiagnosed impaired glucose tolerance/DM was detected. Patients with ongoing hypoglycaemia (II-2, family A and III-2, family F [Fig. 1]) were excluded. Six adults met the criteria but one participant (II-1, family E

[Fig. 1]) was not contactable. Blood glucose concentrations were measured at 0 and 120 min following an oral load of glucose (75 g) in the remaining five adult mutation carriers. The BMI of the adult mutation carriers with normal glucose tolerance ($n=5$) and with DM ($n=8$) was calculated and the means were compared using the Student's t test.

Results

Genetic analysis Seven different heterozygous *ABCC8* mutations were identified in eight probands. Five of the mutations were novel (L1390R, L1431F, Q1459E, A1508P and A1537V) and two have been reported previously in patients with dominantly inherited HH (G1479R and R1539Q) [11]. Each mutation was identified in a single family with the exception of G1479R, which was identified in two unrelated families. All mutations affected residues that are highly conserved across species and occurred within nucleotide-binding domain 2 of SUR1, a previously reported hotspot for dominantly acting mutations. The remaining proband was heterozygous for an in-frame deletion (I284del) in the *KCNJ11* gene.

Of the 35 adult family members (including 18 parents) and eight siblings screened for the identified mutation, 16 adults and five siblings tested positive. This included eight parents, of whom seven were affected with hypoglycaemia or DM. In the remaining case (family C) although the mutation (G1479R) had been inherited from the unaffected father it was shown to co-segregate with HH in the family having been identified in the proband's two affected siblings and paternal cousin. In two families there was no history of hypoglycaemia or DM (Fig. 1, family I and J). Confirmation of family relationships by microsatellite analysis demonstrated that the L1390R and G1479A mutations had arisen de novo in the proband.

Functional analysis of mutant channels The R1539Q and G1479R *ABCC8* mutations have been characterised previously by Pinney et al. [11] and hence these mutations were excluded from functional studies. The ^{86}Rb flux assay showed that the channels with the mutant proteins (SUR1 [A1508P, L1431F, L1390R, Q1459E or A1537V] or Kir6.2 [I284del]) were non-functional as evidenced by the lack of ^{86}Rb efflux on exposure to the channel agonist diazoxide or metabolic inhibition (Fig. 2a–g). Production of the mutant SUR1 proteins L1431F, L1390R, Q1459E and A1537V did not differ from the wild-type SUR1 protein on western blot analysis (Fig. 2h).

Clinical characteristics of children with HH The clinical characteristics of the 14 children with HH (nine probands and five affected siblings) are summarised in Table 1. The

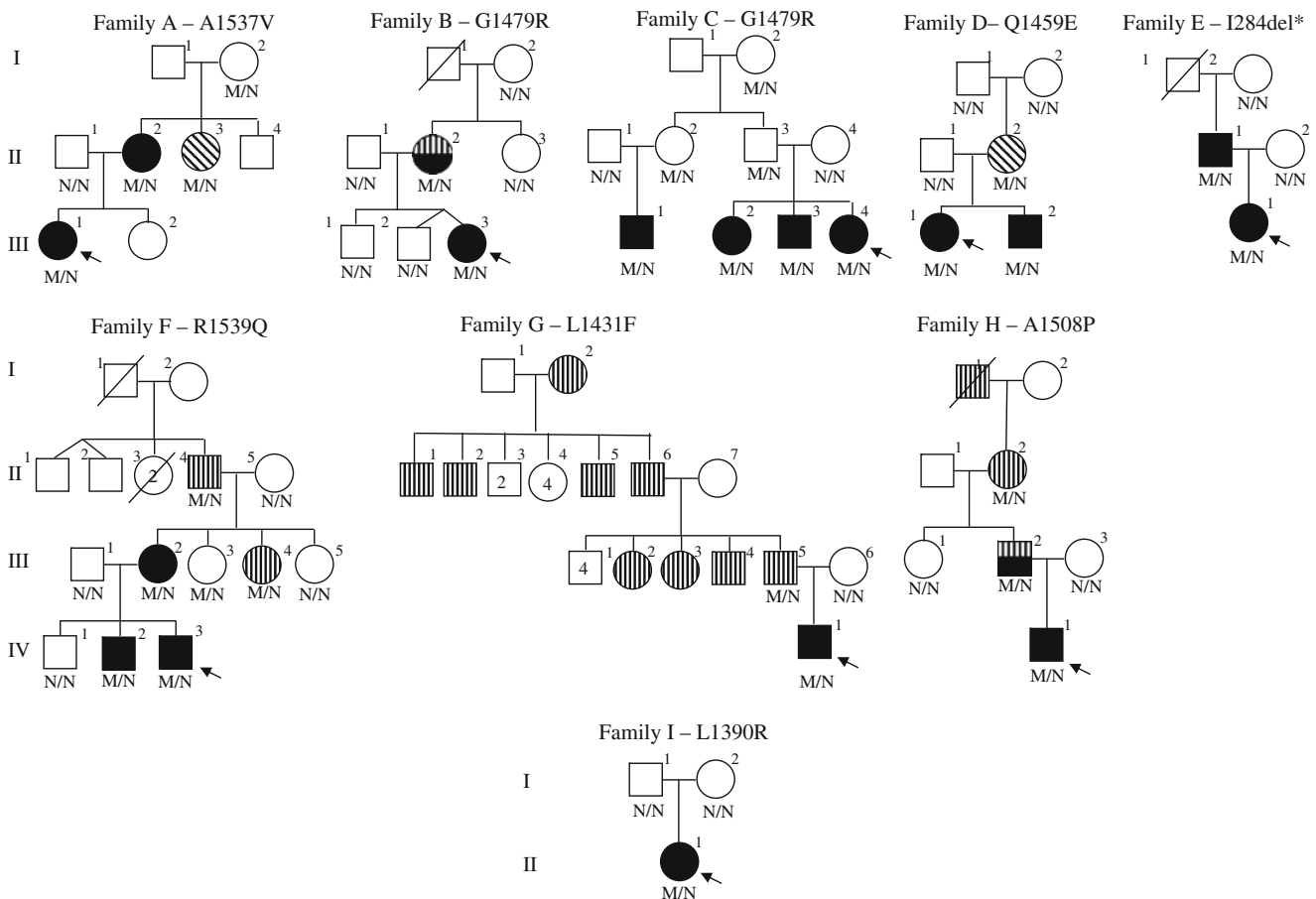


Fig. 1 Partial pedigrees showing inheritance of the dominant mutations in the ten families. Circles represent females and squares indicate males. An arrow indicates the proband. Vertical hatching denotes diabetic individuals and diagonal hatching represents individuals with GDM. Black symbols denote children/adults with hypoglycaemia. Individuals who progressed from hypoglycaemia to

diabetes are indicated by symbols that are half-filled and half-hatched vertically. The genotype is given below each symbol. M/N denotes a heterozygous *ABCC8/KCNJ11* mutation and N/N a normal genotype. The *KCNJ11* mutation is indicated by *; the remaining are *ABCC8* gene mutations

median age at presentation was 1 day and median birthweight was +2.21 SD score (SDS). Hypoglycaemia resolved within 1 week in one case but the remaining children (aged 1–11 years) received ongoing diazoxide treatment.

Phenotype of the adult mutation carriers The median birthweight of the adult mutation carriers ($n=16$; data available for ten individuals) was high at +1.39 SDS (ranging from −2.11SDS to +2.45SDS, Table 2). Interestingly, five of the six adult mutation carriers who were macrosomic at birth developed hypoglycaemia. Two of these had persisting hypoglycaemia at 28 and 39 years of age (Fig. 1: family A, II-2 and family F, III-2), whilst one had a history of transient neonatal hypoglycaemia (Fig. 1: family E, II-1) (Table 2). Two other adults had a past history of hypoglycaemia that progressed to DM (family B II-2 and family H III-2).

Of the remaining 11 adult mutation carriers, there was a history of diabetes (gestational or other) in six further

individuals (family A, II-3; family D, II-2; family G, III-5; family F, II-4 and III-4; and family H, II-2). The remaining five adults (family A I-2, family C I-2, II-2, II-3 and family F III-3) had no history of hypoglycaemia/DM. In total, 5/16 adult mutation carriers had or had had hypoglycaemia and eight either had gestational diabetes (GDM) in multiple pregnancies or were currently diabetic. The age at diagnosis of DM ranged from 20 to 50 years with a median of 31 years (see Table 2). None of the 19 adult family members (aged 20–68 years, median 40 years) who did not carry the *ABCC8/KCNJ11* mutation were known to be affected with diabetes (Fig. 1).

According to the WHO criteria none of the five asymptomatic adult mutation carriers investigated had impaired fasting glucose/impaired glucose tolerance or DM. There was no significant difference in the mean BMI of the adult mutation carriers who developed DM from those who did not ($p=0.7$).

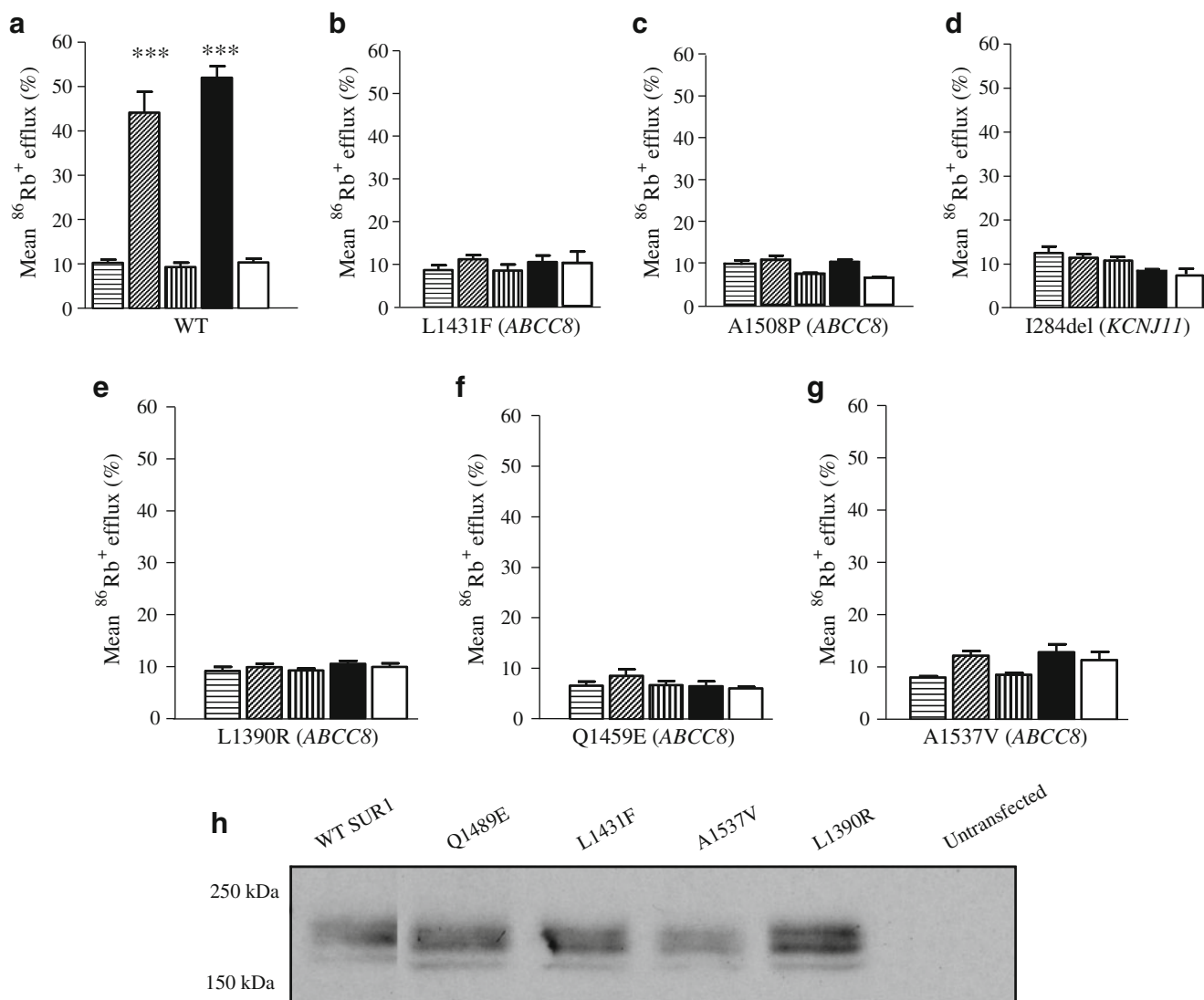


Fig. 2 **a–g** Effects of SUR1/Kir6.2 mutants on K_{ATP} channel function studied with ^{86}Rb efflux. The ^{86}Rb efflux was assayed 48 h after transfection in control cells (horizontal hatching, no drugs), cells treated with 100 $\mu\text{mol/l}$ diazoxide (diagonal hatching), cells treated with 100 $\mu\text{mol/l}$ diazoxide plus 10 $\mu\text{mol/l}$ glibenclamide (vertical hatching), cells treated with 2.5 mmol/l NaCN and 20 mmol 2-deoxy-D-glucose (black) and cells treated with 2.5 mmol/l NaCN, 20 mmol/l 2-deoxy-D-glucose and 10 $\mu\text{mol/l}$ glibenclamide (white). **(a)** Wild

type; **(b)** L143F (*ABCC8*); **(c)** A1508P (*ABCC8*); **(d)** I284del (*KCNJ11*); **(e)** L1390R (*ABCC8*); **(f)** Q1459E (*ABCC8*); **(g)** A1537V (*ABCC8*). Data represent means \pm SEM for three different experiments performed in triplicate ($n=9$); *** $p<0.001$. **h** Western blot analysis of SUR1 shows that the production of the mutant SUR1 proteins L1431F, L1390R, Q1459E and A1537V did not differ from the WT SUR1 protein (177 kDa). WT, wild type

Discussion

This case series reports the phenotype of eight (six novel) dominant *ABCC8/KCNJ11* mutations. We identified 30 individuals (14 children and 16 adults) with heterozygous K_{ATP} channel mutations from ten families. Nineteen of the 30 (63%) had a past/current history of hypoglycaemia and eight adults (50%) had a history of gestational diabetes in multiple pregnancies or were currently diabetic.

The hyperinsulinaemic phenotype of these mutations was mild and diazoxide responsive, as reported in the

literature previously [9, 11, 12]. Of the 14 children, 13 required treatment with diazoxide, whilst the remaining had transient hypoglycaemia that remitted in the neonatal period. These included a pair of siblings (family F [Fig. 1]) heterozygous for the R1539Q mutation in the *ABCC8* gene who had hypoglycaemia for different durations. The older sibling required brief treatment with intravenous glucose after birth while the younger sibling continued to require diazoxide therapy at 3 years of age, demonstrating the variability in the phenotype of HH within the same family.

Table 1 Clinical characteristics of the 14 children with HH

Family	Individual/sex	Mutation/gene	Birthweight SDS	Age at presentation	Diazoxide responsive	Length of treatment with diazoxide
A	III-1/female	A1537V (c.4610C>T)/ <i>ABCC8</i>	+4.66	1 day	Yes	Continuing at 4 years
B	III-3/female	G1479R (c.4435G>A)/ <i>ABCC8</i>	+1.37	1 day	Yes	Continuing at 11 years
C	III-1/male	G1479R (c.4435G>A)/ <i>ABCC8</i>	+2.82	1 day	Yes	Continuing at 1.4 years
C	III-2/female	G1479R (c.4435G>A)/ <i>ABCC8</i>	+1.65	2 days	Yes	Continuing at 8.5 years
C	III-3/male	G1479R (c.4435G>A)/ <i>ABCC8</i>	+2.23	2 days	Yes	Continuing at 5.1 years
C	III-4/female	G1479R (c.4435G>A)/ <i>ABCC8</i>	+1.93	2 days	Yes	Continuing at 1.5 years
D	III-1/female	Q1459E (c.4375C>G)/ <i>ABCC8</i>	+3.14	1 day	Yes	Continuing at 5.8 years
D	III-2/male	Q1459E (c.4375C>G)/ <i>ABCC8</i>	+2.52	1 day	Yes	Continuing at 6 weeks
E	III-1/female	I284del (c.850_852del)/ <i>KCNJ11</i>	+1.48	1 day	Yes	Continuing at 1.8 years
F	IV-3/male	R1539Q (c.4616G>A)/ <i>ABCC8</i>	+2.19	1 day	Yes	Continuing at 3.2 years
F	IV-2/male	R1539Q (c.4616G>A)/ <i>ABCC8</i>	+0.07	1 day	NA	NA
G	IV-1/male	L1431F (c.4291C>T)/ <i>ABCC8</i>	−0.98	1 year	Yes	Continuing at 2.5 years
H	IV-1/male	A1508P (c.4522G>A)/ <i>ABCC8</i>	+4.09	1 day	Yes	Continuing at 9.2 years
I	II-1/female	L1390R (c.4619T>G)/ <i>ABCC8</i>	+3.22	1 day	Yes	Continuing at 11 years

NA, not applicable, as had transient hypoglycaemia that did not require treatment with diazoxide

Table 2 Summary of the clinical features of the adult mutation carriers

Family	Patient/ethnicity	Current age (years)	BMI (kg/m ²)	BW SDS	Hypoglycaemia/past or current	Diabetes ^a	Treatment ^b	OGTT blood glucose ^b (mmol/l)	
								Fasting	2 h
A	II-2/white British	28	21	+1.42	Yes, current	NA	Nil	NA	NA
A	II-3/white British	30	26.2	+1.23	No	Yes, GDM at 26 and 29 years	Dietary	NA	NA
A	I-2/white British	57	21	–	No	No	NA	4.0	5.3
B	II-2/white British	44	33	+1.4	Yes, past	Yes, GDM at 23 years, overt diabetes at 30 years	Insulin (metformin tried initially)	NA	NA
C	I-2/white Irish	73	32.2	–	No	No	NA	4.9	4.9
C	II-2/white Irish	32	31.4	+0.35	No	No	NA	4.8	7.2
C	II-3/white Irish	37	29.6	−2.11	No	No	NA	5.4	4.4
D	II-2/white British	30	23	–	No	Yes, GDM at 21, 23 and 25 years	Insulin during pregnancies	NA	NA
E	II-1/white British	20	–	+2.07	Yes, past	Not known	i.v. glucose for neonatal hypoglycaemia	Not known	Not known
F	II-4/white Irish	72	23.7	–	No	Yes, 47 years	Metformin+ gliclazide	NA	NA
F	III-2/white Irish	39	21.6	+2.45	Yes, current	NA	Nil	NA	NA
F	III-3/white Irish	35	25.1	−0.39	No	No	NA	3.7	7.0
F	III-4/white Irish	40	42.2	−0.58	No	Yes, GDM at 36 years, overt diabetes at 39 years	Metformin	NA	NA
G	III-5/black African	43	24.9	–	No	Yes, 20 years	Insulin	NA	NA
H	II-2/white British	65	28.4	–	No	Yes, 50 years	Insulin	NA	NA
H	III-2/white British	40	31.1	+1.38	Yes, past	Yes, 38 years	Metformin	NA	NA

^a Yes/no in the case of normal OGTT and, if yes, age of diagnosis is shown; NA if current hypoglycaemia

^b If applicable

BW, birthweight; NA, not applicable; Nil, hypoglycaemia not requiring treatment with drugs

Of the 16 adult mutation carriers, two were recognised to have had hypoglycaemic episodes after the diagnosis of HH in their children. Three others had a previous history of hypoglycaemia; of these individuals, one had had transient neonatal hypoglycaemia treated with intravenous glucose, while the other two had had more persistent hypoglycaemia that remitted in young adulthood. The remaining adult mutation carriers (11/16) had no current/past history of hypoglycaemia. This is consistent with the study by Pinney et al. in which 14/29 adults were asymptomatic [11]. The incomplete penetrance of hypoglycaemia is not mutation specific. Nevertheless, in utero hyperinsulinism was evident from the higher birthweights of the mutation carriers. Hence, these dominant K_{ATP} mutations result in a varied phenotype, ranging from asymptomatic macrosomia to persistent HH requiring diazoxide treatment. In contrast to the paper by Huopio et al. [9], in which all the patients inherited their mutation from their mothers, our probands inherited the mutations from either the maternal or paternal allele, refuting the possibility of a parent-of-origin effect or an imprinting defect.

It has been debated whether dominant K_{ATP} channel mutations causing HH predispose to the development of DM in adulthood [9, 11, 14, 18]. Huopio et al. first reported a dominant inactivating *ABCC8* mutation, E1506K (E1507K according to reference sequence NM_000352.2), that caused HH progression to hypoinsulinaemic DM during middle age [9, 14]. In their study, adults heterozygous for this particular mutation showed reduced insulin secretory capacity and only 1/11 adults had normal glucose tolerance [14]. In addition, the individuals who developed DM had reduced insulin sensitivity (3/4 individuals with DM were overweight). The authors hypothesised that the primary insulin secreting defect may be related to beta cell apoptosis due to raised intracellular calcium concentrations and that the accompanying insulin resistance may be the reason for conversion from impaired glucose tolerance to DM. Another family with this mutation has recently been reported with HH in childhood and later-onset diabetes [19]. Interestingly, insulin deficiency is observed in the beta cell-specific dominant-negative Kir6.2 mouse [20]. Furthermore, Abdulhadi-Atwan et al. described a patient with HH due to a de novo *ABCC8* mutation who presented with DM at 10.5 years of age [12]. This child was obese (BMI 30.2 kg/m²), supporting the hypothesis that insulin resistance in conjunction with a dominant K_{ATP} channel mutation can predispose to the development of DM.

However, a recent study that looked at the prevalence of DM in a large number of families with dominant K_{ATP} channel mutations [11] reported diabetes in just 13.8% of the adult carriers. The prevalence of DM in this cohort was comparable with the estimated prevalence of type 2 DM in adults in the USA, which ranges from 9.6% to 21% [21]. In

contrast we identified two adults who developed DM in adulthood following hypoglycaemia in early life and six adults who were diagnosed with either GDM or overt diabetes before the age of 50 years. Excluding the two adults who had symptoms of hypoglycaemia at the time of the study, the prevalence of DM (gestational or other) was high, with 8/14 adult mutation carriers being affected in our cohort. This may even be an underestimate as some individuals who do not have DM may go on to develop it later. In contrast, none of the 19 adult non-carriers of the *ABCC8/KCNJ11* mutation within these families were known to be diabetic.

The difference in the reported prevalence of diabetes in the adult mutation carriers in our study and that of the US study [11] can be partly explained. First, there were a high percentage of adults with ongoing hypoglycaemia (51%) in the American study. The presence of hypoglycaemic symptoms in these adults signifies the persistence of hyperinsulinism. Surprisingly, these adults were not excluded from the final analysis of the adults affected with DM. Second, adults with GDM were excluded from the final reporting of patients with DM. The authors argue that the development of GDM in adults with a dominant K_{ATP} channel mutation may not have the same implication for the eventual development of diabetes as it may reflect impaired glucose-stimulated insulin release due to a direct consequence of defective K_{ATP} channel activity [11]. However, GDM per se forms an important diagnosis and has implications for the management of the individual during her pregnancy and for the growing fetus. Hence, we believe that the increased risk of GDM merits reporting in this study.

However, it is also clear that not all adult mutation carriers develop DM. We confirmed normal glucose tolerance in five of the adults who had no history of DM. In our cohort, the mean BMIs of those with normal glucose tolerance ($n=5$) and those with DM ($n=8$) were not significantly different. Nevertheless, the adults who developed overt diabetes following GDM in multiple pregnancies did have higher BMIs (33 and 42.2 kg/m²) than those adults who did not develop overt diabetes following GDM (23 and 26 kg/m²). However, these data must be interpreted with caution as the number of individuals in each group was small. In addition, patients with the same G1479R mutation had varied glucose tolerance, suggesting that the development of DM is not mutation specific and other environmental/genetic factors may be involved.

This study confirms that dominant *ABCC8/KCNJ11* mutations can lead to a variable phenotype of neonatal HH, impaired glucose intolerance and diabetes mellitus later in life. The mechanistic explanation for the neonatal HH involves subtle defects in the function of K_{ATP} channels. We carried out Rb flux assays to prove the

pathogenicity of the novel mutations identified in the study. The homozygous in vitro model was not stimulated by the K_{ATP} channel agonist diazoxide, a finding which is consistent with these mutations being disease causing. Western blotting confirmed that the mutant SUR1 proteins were produced in the cell (Fig. 2b). In patients with dominant *ABCC8/KCNJ11* mutations, K_{ATP} channels are assembled from mutant and wild-type SUR1 and KIR6.2 subunits (channel hetero-octomers are formed with zero to four SUR1 and KIR6.2 mutant subunits). The eventual K_{ATP} channel activity is based on the number of mutant subunits present (in the complex consisting of both mutant and wild-type subunits) and the extent to which the mutant subunit exerts its adverse effect on channel activity. The resulting impairment in channel activity is sufficient to activate the downstream voltage-gated calcium channels and thus trigger unregulated insulin secretion. These patients respond to diazoxide presumably because some wild-type SUR1 subunits are present and allow diazoxide activation. The milder phenotype of dominant mutations reflects the subtle K_{ATP} channel defect in comparison with recessive mutations, which lead to defects in channel biogenesis, trafficking and assembly.

The mechanism of glucose intolerance and diabetes mellitus in patients with dominant *ABCC8/KCNJ11* mutations is not so clear at present. Possible explanations include a progressive failure in beta cell function due to ‘exhaustion’, increased beta cell apoptosis as a result of raised intracellular calcium concentration and the influence of other genetic or environment factors. Further studies are required to understand how these mutations cause glucose intolerance and diabetes later in life.

Conclusion

Dominant mutations in *ABCC8/KCNJ11* cause a varying phenotype ranging from asymptomatic macrosomia to persistent HH in childhood. There is an increased risk of DM in the adult mutation carriers. This has important implications for follow up of children with dominantly acting *ABCC8/KCNJ11* mutations and the management of the adult carriers.

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